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**Effects of *Bacillus thuringiensis*
and Defoliation by Gypsy Moth
Larvae on Nontarget Aquatic
Arthropods**

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Effects of *Bacillus thuringiensis* and Defoliation by Gypsy Moth Larvae on Nontarget Aquatic Arthropods

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Abstract

Impacts of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) and gypsy moth defoliation on nontarget aquatic arthropods were evaluated in eastern West Virginia during 1991 and 1992. Aquatic arthropods were collected from riffles using a Portable Invertebrate Box sampler or a Surber sampler and from leaf packs in pools using a D-ring net. Analysis of Variance (ANOVA) were performed on abundance and biomass for the most abundant orders, families, and genera. Of 17 genera analyzed, only two differed significantly among treatments, with only one, the mayfly, *Epeorus*, showing significant decreases in defoliation and *Btk* streams.

Introduction

The gypsy moth (*Lymantria dispar*) has become the most important defoliating pest of broadleaved forests in the eastern United States. To suppress populations of this pest, federal and state agencies use the insect growth regulator, diflubenzuron, or the bacterial insecticide, *Bacillus thuringiensis* (*Bt*). In recent years, the use of *Bt* has been increasing.

Bacillus thuringiensis is a naturally occurring soil bacterium. The variety used for gypsy moth suppression, *Bacillus thuringiensis* var. *kurstaki* (*Btk*), is believed to be primarily toxic to Lepidoptera larvae. Mortality occurs after larvae have ingested foliage on which both *Btk* spores and the parasporal crystals have been deposited (Boberschmidt et al 1989).

Because of the apparent specificity of *Btk* for Lepidoptera, it is commonly used in the vicinity of sensitive aquatic sites to avoid the negative impacts on nontarget aquatic organisms associated with the application of diflubenzuron (Ali and Lord 1981). In addition, other varieties of *Bt*, *Bt* var. *israelensis* in particular, are used to control the aquatic larvae of mosquitoes (Diptera: Culicidae) and blackflies (Diptera: Simuliidae).

There is little information on the effects of *Btk* on aquatic arthropods. Kreutzweiser et al (1992) reported minimal effects of *Btk* on aquatic organisms; abundance of two mayfly (Ephemeroptera) genera, and one stonefly (Plecoptera) was reduced by 7% to 15%. In another study of the effects of *Btk* on aquatic arthropods, Eidt (1985) documented reduced abundance only for two blackfly genera. Effects for some Plecoptera and Trichoptera were suggested, but were confounded because of the time lag between treatment and mortality and the lack of correlation to *Btk* concentration (Eidt 1985).

The alternative to *Btk* application is to allow defoliation by gypsy moth larvae to occur. While defoliation may directly affect hydrology and water quality, aquatic invertebrates are not known to be at significant risk from these effects (USDA 1989). Defoliation can allow more sunlight to reach forest streams, resulting in increased water temperatures. This may adversely influence water chemistry and life functions of some aquatic organisms. Greater insolation may be mitigated by refoliation that usually occurs before summer stream temperatures become critical in July and August. Also, impacts may be lessened because Appalachian streamside vegetation often contains a significant component of rhododendron and yellow-poplar, species avoided by the gypsy moth (USDA 1989).

In this study, we evaluated the effects of *Btk* and defoliation by gypsy moth larvae on aquatic arthropods. Biomass (weight of invertebrates) and abundance (numbers of individuals) of aquatic insects from streams in watersheds treated with *Btk* and defoliated by gypsy moth larvae were compared to those from streams in unsprayed, undefoliated watersheds.

Methods

Study Sites -- Seven streams, located in Grant and Pendleton counties, West Virginia, were monitored in 1991 and 1992. These streams were similar; all flowed eastwardly, were limestone based, and were located in forests dominated by oak (*Quercus sp.*), eastern hemlock (*Tsuga canadensis*), and maple (*Acer sp.*). All streams were perennial except one, which was intermittent in 1991.

The streams were allocated among the treatments in the following manner: two streams were located in watersheds on which *Btk* was applied, three were in watersheds that received defoliation, and two were in watersheds that were neither sprayed nor defoliated.

Treatment -- *Btk* was applied to the treatment watersheds on May 10, 1991. The treatment consisted of undiluted Foray 48B, applied in one application at a rate of 96 oz./acre and a dosage of 36 BIU/acre. No *Btk* application was made in 1992.

Sampling Methods -- On each main stream channel, three 100 ft. plots were delineated. Each plot was sampled eight times at bi-weekly intervals during the springs and summers of 1991 and 1992. Two pre-spray samples were taken prior to mid-May 1991. Sampling occurred from April 20 to August 6, 1991, and April 17 to August 5, 1992. In 1992, the streams were sampled within two or three days of the 1991 sampling dates.

Invertebrates were sampled from leaf packs in pools using a D-ring aquatic net (Figure 1). All insects were removed from the leaf packs, and the leaves were dried and weighed in the laboratory. Riffles were sampled using either a PIBS (Portable Invertebrate Box sampler) (Figure 2) or a Surber sampler (Figure 3). The PIBS was used when water depth precluded the use of the Surber sampler. All invertebrates were identified to order, family, and genus (if possible), and body lengths were measured. Biomass of aquatic invertebrates was estimated using the length-weight regression equations of Smock (1980).

Analyses

Simple, one-way ANOVA were performed on the abundance and biomass of individuals in riffles and in leaf packs from pools. A sufficient sample size for analysis was assumed if >100 individuals/taxa were collected in the combined riffle samples from 1991 and 1992. For leaf pack samples, sufficient sample size for analysis was assumed if >10 individuals/taxa were found in a 5g sample. If the overall F-test was significant ($P \leq 0.05$), Least Square Means comparisons were performed to determine which means were different.

LS Means comparisons were required because of the unbalanced design (i.e., two control, three defoliation, and two spray treatment groups) (SAS 1989).

Results

During the two years of stream monitoring, 39 genera were collected. Of 17 genera in sufficient abundance to be analyzed, two were significantly affected.

Analyses were restricted to within-year comparisons only. Between-year comparisons could not be performed because of the influence of weather conditions in 1991 and 1992. National Oceanographic and Atmospheric Administration (NOAA) data indicated significant between-year differences for both temperature and precipitation; 1991 was warmer and with lower precipitation than 1992.

Abundance -- Significant among-treatment differences in abundance in riffle samples were observed for only one genus (Table 1). Abundance of *Epeorus* (Ephemeroptera: Heptageniidae) in both 1991 and 1992 was significantly greater (ave. 5 fold) in the control streams than in the defoliation or spray streams. *Epeorus* numbers in defoliation and spray streams were not different in either year. No significant differences were observed for any other taxa collected in riffle samples in 1991 or 1992.

In leaf packs from pools, *Pycnopsyche* (Trichoptera: Limnephilidae), abundance was significantly greater in sprayed streams than in either control or defoliation streams in 1992 (Table 1).

Biomass -- In 1991, the biomass of *Epeorus* from riffle samples was significantly greater (ave. 6 fold) in control streams than in either sprayed or defoliation streams; defoliation and spray streams were not different (Table 1). No differences in *Epeorus* biomass were observed in 1992. For *Pycnopsyche*, no differences in biomass were observed among treatments in 1991. However, in 1992, biomass of *Pycnopsyche* was significantly greater in defoliation and spray streams than in control streams.

Discussion

Similar to other studies evaluating the effects of *Btk* on aquatic arthropods, we observed few significant effects. In addition, effects of defoliation by gypsy moth larvae were found to be minimal. Of 17 genera analyzed, only two differed significantly among treatments, with only one, the mayfly, *Epeorus*, showing significant decreases in both defoliation and *Btk* streams. The other, the caddisfly, *Pycnopsyche*, appeared to benefit from *Btk* application.

Among other mayfly genera, we found no treatment effects for the genus *Paraleptophlebia* (Leptophlebiidae). In contrast, Eidt (1985) found that while *Leptophlebia* (Leptophlebiidae) showed no immediate effects of *Btk*, more adults emerged from the controls and low concentrations of *Btk* than from higher *Btk* concentration treatments. *Paraleptophlebia* did not appear to show any such effects. In fact, abundance was highest in the sprayed streams.

While we observed no adverse effects among Trichoptera resulting from either *Btk* application or defoliation, Eidt (1985) reports a possible effect of *Btk* on the genus *Chimarra* (Trichoptera: Philopotamidae). Reduced abundance was observed, but was not considered significant because it took 12 or more days for the test specimens to die in the treatments, and these deaths could not be correlated to the concentration of *Btk* used (Eidt 1985). Another genus of caddisfly, *Hydropsyche* (Hydropsychidae), did well in *Btk* treated water, but no relationship to *Btk* concentration was found (Eidt 1985).

In our study, the caddisfly, *Pycnopsyche* (Limnephilidae), was more abundant in sprayed streams than in streams in control or defoliated watersheds. This may be an artifact of *Btk* protecting the foliage, making more leaves available to fall into the stream at leaf drop. Because *Pycnopsyche* build leaf cases after hatching in autumn (Wiggins 1978), additional foliage may have improved overwinter survival.

The lack of effects among Diptera is contrary to observations by Eidt (1985). He found that *Btk* application reduced the abundance of *Simulium* and *Prosimulium* (Diptera: Simuliidae). While we observed no *Btk* effects, a possible defoliation-related effect was observed for the Simuliidae. A non-significant increase in abundance was observed in streams in defoliated watersheds between 1991 and 1992. This may have resulted from an increase of diatoms in the stream because of increased sunlight, or it may be from a delayed hatching of blackfly eggs as a result of the 1991 drought.

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Table 1. Summary of Effects of *Btk* Application and Defoliation on Aquatic Arthropods, West Virginia, 1991 and 1992.

Order	Family	Genus	Abundance	Biomass
Ephemeroptera	Baetidae	Baetis	ND ^a	ND
	Ephemerellidae	Ephemerella	ND	ND
	Heptageniidae	Epeorus	Control>Defol= Spray both 1991 and 1992	Control>Defol= Spray 1991 only
		Heptagenia	ND	ND
		Stenonema	ND	ND
	Leptophlebiidae	Paraleptophlebia	ND	ND
	Siphonuridae	Ameletus	ND	ND
Plecoptera	Chloroperlidae	Chloroperla	ND	ND
	Perlidae	Acroneuria	ND	ND
	Pteronarcyidae	Pteronarcys	ND	ND
	Peltoperlidae	Peltoperla	ND	ND
Trichoptera	Hydropsychidae	Potamyia	ND	ND
	Rhyacophilidae	Rhyacophila	ND	ND
	Limnephilidae	Pycnopsyche	Spray>Control= Defol 1992 only	Spray=Defol> Control 1992 only
	Brachycentridae	Brachycentrus	ND	ND
Diptera	Chironomidae	Pentaneura	ND	ND
	Simuliidae	Prosimulium	ND	ND

^aND = No significant difference observed between treatments

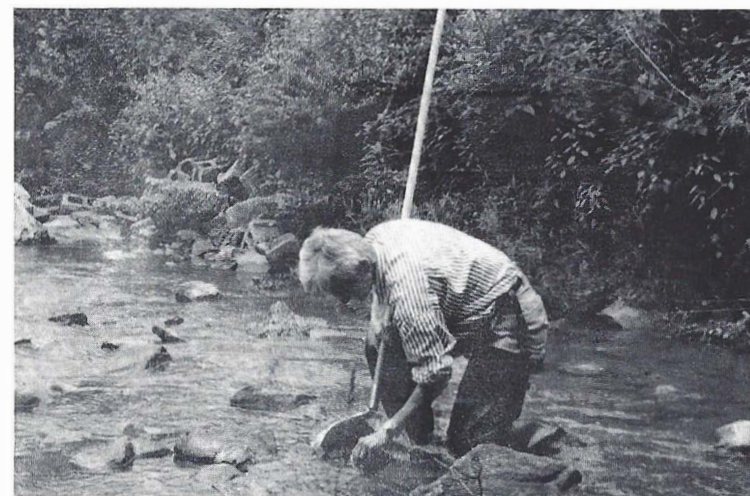


Figure 1. D-Ring aquatic net used for sampling aquatic microinvertebrates.



Figure 2. Portable invertebrate box sampler used for sampling aquatic microinvertebrates.



Figure 3. Surber sampler used for aquatic invertebrates.

Notes

Notes



Pesticide Precautionary Statement

This publication reports the aerial application of insecticides. It does not contain recommendations for insecticide use, nor does it imply that the uses discussed here have been registered. All uses of insecticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

Caution: Insecticides may be injurious to humans, domestic animals, desirable plants, and fish or other wildlife if they are not handled or applied properly. Use all insecticides selectively and carefully. Follow recommended practices for the disposal of surplus insecticides and insecticide containers.

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